**2812-Pos Board B242**
**Promotion of Coalescence in Bicellar Mixtures by an SP-B Fragment**
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Maintenance of a functional surfactant layer requires the transfer of material between bilayer reservoirs and the surface-active layer. It is believed that SP-B facilitates the interlayer contact and mixing implicit in such activity. SP-B's role in promoting these contacts was investigated though the use of bilayered micelle mixtures containing short- and long-chain lipids. Upon warming, these bicellar mixtures progressively coalesce into more extended structures and are thus an interesting system in which to study the capacity of polypeptides to promote interactions between lipid structures. $^{2}$H NMR was used to identify the capacity of perturbation by an SP-B fragment (SP-$B_{63-78}$) upon bicellar mixtures of DMPC-$d_{54}$/DMPG/DHPC and DMPC-$d_{54}$/DMPC/DHPC in nominal 3:1:1 molar ratios. In the presence of mixtures containing anionic lipids (DMPC-$d_{54}$/DMPG/DHPC), SP-$B_{63-78}$ (concentration approximately 10% of lipid weight) was found to lower the temperature at which coalescence to extended lamellar structures occurred. Conversely, when anionic lipids were replaced with zwitterionic lipids (DMPC-$d_{54}$/DMPC/DHPC), SP-$B_{63-78}$ did not perturb the temperature at which the transition to extended lamellar structures occurred. These results indicate that the interaction of SP-$B_{63-78}$ with model membranes is dependent upon the presence of anionic lipids and further suggest a mechanism by which full length SP-B may interact with membranes.

**2813-Pos Board B243**
**Lipid Composition Influences the Insertion and Folding of pHLIP Peptides**
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The study of polypeptide insertion into biological membranes can inform our understanding of membrane protein stability and folding, and has potential practical applications. This has been hard to investigate, since peptides hydrophobic enough to form transmembrane helices are likely to be insoluble in aqueous buffers, and soluble peptides are unlikely to insert. pH-Low Insertion Peptides (pHLIPs) provide an opportunity to study insertion since they are soluble monomers that bind to bilayer surfaces at neutral pH, and can be triggered to form monomeric transmembrane helices in acidic conditions.
This set of unusual properties allows the study of spontaneous insertion and exit of a transmembrane polypeptide by changing the pH of its microenvironment. Previous studies showed that pHLIP inserts into a POPC liposomes through rapid formation of an interfacial helix (~ 0.1s), followed by a slow insertion pathway. The time-course of pHLIP insertion can be changed by varying the number of protonatable groups at the inserting end of the peptide, which need to be moved across the bilayer.
We have employed various biophysical methods: fluorescence spectroscopy, anisotropy, CD, OCD, and stopped-flow fluorescence, to study the pHLIP insertion into bilayers composed of different monounsaturated lipids (diC(14:1)PC, diC(16:1)PC, diC(18:1)PC, diC(20:1)PC, and diC(22:1)PC). We found that pHLIP can form a TM helix in bilayers of different thickness. The kinetics of pHLIP insertion vary, and correlate with bilayer thickness and fluidity. Further, pHLIP association with bilayer surfaces also depends on the lipid composition. The activation energy of pHLIP insertion increases with the membrane thickness but the process of inserted helix formation does not significantly depend the membrane type. A model for membrane-associated insertion/folding is discussed. This work was supported by grants from National Institute of Health, GM073857, and CA133890.

## Protein-Lipid Interactions III

**2814-Pos Board B244**
**Enhanced HIV Fusion Inhibitors Efficacy Requires Membrane Affinity and Exposure of the Pocket Binding Domain of C34 Derivatives**
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Viral fusion inhibitors block the fusion between the membranes of an enveloped virus and a target cell, therefore preventing the entry of the viral content. It has been demonstrated that the combination of cholesterol-tagging and dimerization of the C34 peptide sequence resulted both in an increase of the antiviral potency and extension of the in vivo half-life of two new HIV fusion inhibitors: HIVP3 (C34-$PEG_4$-cholesterol) and HIVP4 ($[C34\text{-}PEG_4]_2$-cholesterol). Given the importance of lipophilicity and selective affinity for different lipid domains, the aim was to evaluate the interaction of these HIV fusion inhibitor peptides with biomembranes model systems and human blood cells, in order to clarify where and how they are located. This study allowed the understanding of the mechanism of action of these peptides at the molecular level, and which strategies may be followed to increase their fusion inhibition efficacy. Membrane partition, dipole potential and surface pressure measurements indicated that HIVP3 and HIVP4 interact preferentially with cholesterol-rich liquid ordered membranes. HIVP3 and HIVP4 are able to interact with human erythrocytes and peripheral blood mononuclear cells to a similar degree as previously described for the simpler drug C34-cholesterol. However, the pocket binding domain(PBD) of both HIVP3 and HIVP4 is more exposed to the aqueous environment than in C34-cholesterol. The efficient blocking of HIV entry results from the synergic effect between the membranotropic behavior and the enhanced exposure of the PBD[1]. Maximizing antiviral activity requires finding the proper balance of membrane affinity and exposure of the peptide moiety, through variations in the lipid-binding domain, PEG spacer region, and number of peptide moieties in the construct.
[1] Augusto MT et al.(2014)Improvement of the HIV fusion inhibitor C34 efficacy by membrane anchoring and enhanced exposure.*J Antimicrob Chemother*,69:1286-1297

**2815-Pos Board B245**
**Strength, Not Depth: An Exploration of Differential Membrane Binding Kinetics of Synaptotagmin-1 and Synaptotagmin-7 C2 Domains**
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Synaptotagmins (Syt) play an essential role in mediating $Ca^{2+}$-dependent vesicle fusion, and are believed to function by binding its $Ca^{2+}$-sensing C2 domains to anionic phospholipids and thereby bring the vesicle near the membrane into which the vesicle will fuse. Many Syt isoforms exist, and despite their close structural similarity, the binding kinetics of each isoform to membranes can vary widely. We have performed an extensive set of molecular dynamics simulations on Syt-1 and Syt-7 C2 domains to characterize the factors that determine the rapid unbinding for Syt-1 and the slow unbinding of Syt-7. Through repeated atomistic binding simulations of Syt-1 and Syt-7 C2 domains to anionic membranes, enabled by our enhanced dynamics membrane mimetic model (HMMM), we found that while the penetration depth of the different loop regions are unchanged, Syt-7 C2 domains form many more contacts with anionic head groups. These additional contacts both strengthen and lengthen duration of the interaction between Syt-7 and the membrane, including contacts between loop 2 of Syt. These results provides the first atomistic picture of differential binding of different isoforms of Syt.

**2816-Pos Board B246**
**How Synaptotagmin I, N-BAR and F-BAR Domains Generate Membrane Curvature**
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Protein-induced membrane curving governs many cellular processes, including cell division, growth and cell-cell communication. In order to unravel the mechanism for how membrane curvature occurs driven by the proteins, one needs to have detailed molecular pictures on the key membrane-protein interactions and protein-protein interactions. Computer simulation serves as a great tool in providing such molecular pictures and thus in rationalizing how proteins curve membrane. For example, it is known that neurotransmitter release involves a Ca++ ion-regulated fusion process between synaptic vesicles and the presynaptic membrane, but little is known for how such small ions function. With the help of computer simulation, we realized the Ca++-sensor protein, synaptotagmin I, undergoes a conformational transition after binding to both the Ca++ and membrane that differs from the membrane-free crystal/NMR structure. The new synaptotagmin conformation has its C-terminal helix to interact with the presynaptic membrane, and thereby, causes the presynaptic membrane to curve and facilitates membrane-vesicle fusion. Another example in showing the power of computer simulation lies in resolving how N-BAR and F-BAR domains sculpt a flat membrane. As these domains form a lattice in the sculpting process, we identified the optimal lattice type and key protein-protein interactions within the lattice. The whole process of membrane tubulation from a flat membrane was resolved from the simulations, and agreements were found between the lattice structures observed via cryo-electron microscopy and the simulations.